PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
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Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 26 August 1997 (26.08.97)	
International application No. PCT/GB97/00074	**Applicant's or agent's file reference** JDM/P93928WO
International filing date (day/month/year) 10 January 1997 (10.01.97)	**Priority date** (day/month/year) 10 January 1996 (10.01.96)
Applicant RUDLAND, Philip, Spencer et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

07 August 1997 (07.08.97)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
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Facsimile No.: (41-22) 740.14.35

Authorized officer

S. De Michiel

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3

PATENT COOPERATION TREATY

REC'D 10 MAR 1998
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PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
JDM/DCS/P.93928WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB97/00074	10/01/1997	10/01/1996
International Patent Classification (IPC) or national classification and IPC		
C12Q1/68		
Applicant		
THE UNIVERSITY OF LIVERPOOL et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 15 sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☒	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07/08/1997	06.03.98
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Hoesel, H Telephone No. (+49-89) 2399-8693

Form PCT/IPEA/409 (Cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB97/00074

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

2,4-11,18,20-23	as originally filed			
1,1a,3,12-17,19	as received on	19/12/1997	with letter of	16/12/1997

Claims, No.:

1-17	as received on	19/12/1997	with letter of	16/12/1997

Drawings, sheets:

1/8-8/8	as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

see separate sheet

4. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:

see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1,2, 4 - 6, 8 - 14, 16
	No:	Claims	7, 15, 17
Inventive step (IS)	Yes:	Claims	6, 8 - 13
	No:	Claims	1, 2, 4, 5, 7, 14 - 17
Industrial applicability (IA)	Yes:	Claims	1, 2, 4 - 17
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

SECTION I:

1. The arbitrarily chosen length of DNA fragments as given in claim 3 and 7 has no support in the application documents as filed. According to p. 6 lines 18 only fragment lengths of 1300 - 1500 bp are supported. Claims 3 and 7 do therefore not meet the requirements of Art. 41(2) PCT.

   As the unsupported length is the only technical feature of claim 3, this claim has not been examined with respect to novelty and inventive step.

2. Claim 17 has been generalized in a manner contravening the requirements of Art. 41(2) PCT. Claim 17 now generally pertains to a medicament "adapted to target a regulatory (metastasis inducing) DNA...", which might include, besides nucleic acids capable of hybridization, other compounds such as DNA binding proteins or intercalating agents. Claim 17 as originally filed was however limited to nucleic acid compounds.

SECTION VIII:

3. The term "regulatory DNA" may be interpreted as to concern (i) regulatory, non-translated regions of the DNA or (ii) regulatory genes. Thus, the scope of claims 7 and of 15 - 17 insofar as dependent upon claim 7 is obscure contrary to Art. 6 PCT.

4. If the term "regulatory DNA" is to be interpreted in the first sense, Claim 1 lacks clarity due to an inherent inconsistency since the screening method is defined in terms of a randomly obtained result. It is evident (as will be discussed in Section V that the screening method is suitable to identify all metastasis inducing DNA - species, whether these are expressed or not.

5. The term "tagged (DNA) fragments" is also open to interpretation rendering the scope of the claim 1 obscure, contrary to Art. 6 PCT.

   Usually tagging serves for identification of particular analytes. In this sense, a tag might include specific sequences present in the transfected DNA (of up to 50kb

length, see claim 2) such as human specific ALU repeats. The special meaning that is given to the term "tag" with respect to its purpose and function by the description (p. 3, lines 19 - 22, the sentence extending between p 6 and 7) is not mentioned in claim 1.

SECTION IV:

6. The independent claims are not so linked as to form a single general inventive concept (Rule 13.1 PCT) for the following reasons:

While claims 8 - 14 relate to regulatory, apparently untranslated DNA sequences,, claim 14 generically concerns the use of a structural gene as a metastasis inducer. The common concept linking together the subject-matters of claims 8 - 13 on one hand and claim 14 on the other may thus be formulated as the presence of metastasis inducing nucleic acids comprising expressed or non-expressed regulatory sequences. However, a variety of structural genes (e.g. oncogenes) that are capable of inducing or promoting metastasis, and thus the common technical link is already known (see the novelty objection against claim 7, item 7, Section V).

The use according to Claim 14 is furthermore not technically linked in the sense of same or corresponding technical features with the screening method according to claim 1.

The application thus contains the following separate groups of inventions:

1. "Regulatory", metastasis inducing DNA and method of identifying such sequences (including regulatory structural genes, see Section VIII, item); claims 1 - 7.

2. Regulatory, non-translated DNA fragments capable of inducing metastasis (no open reading frames) and correlated with increased osteopontin expression, claims (8 - 13)

3. use of an osteopontin gene as a metastasis inducer, claim 14.

SECTION V:

Reference is made to the following documents:

D1: WO-A-86/03226
D2: WO-A-94/28129
D3: B.R.Davies et al, Cancer Res **54**, 1994, p. 2785-93
D4: E.I.Behrend et al, Cancer Res **54**, 1994, p. 832-7

The applicant has submitted the documents D5 = H.Chen et al, Oncogene vol.14, 1997, p. 1581 - 1588 for technical information.

7. A number of genes or DNA sequences associated with the induction of metastases, such as osteopontin or various oncogenes (e.g. c-myc, ras variants), is known in the state of the art (cf. D2, claims 1 and 2).

Thus, the subject-matter of claim 7 in its broadest interpretation lacks novelty, contrary to Art. 33(2) PCT.

8. Oligonucleotide fragments of such oncogenes (for use as probes or primers) are known in the state of the art. For the reasons discussed in items 3 and 7, also the subject-matter of claims 15 - 17 is not sufficiently limited with respect to this state of the art.

Consequently, claims 15 and 17 lack novelty (Art-. 33(2) PCT), claim 16 lacks inventive step (Art. 33(3) PCT).

9. The subject-matter of claims 8 - 13 which concern several distinct DNA fragments which appear to represent regulatory regions of genomic DNA and the presence of which is correlated with increased expression, of osteopontin is neither disclosed nor rendered obvious by the prior art taken into consideration.

Claims 8 - 13 thus satisfy the requirements of Art. 33(2) and (3) PCT.

10. Claims 1, 2, 4 and 5 do not meet the requirements of Art. 33 (3) PCT.

A screening method based on transformation of a benign tumorigenic cell line with DNA fragments obtained from a human metastatic cancer specimen, transfection into a host animal, and recovery of the human DNA from metastases produced by the host animal is disclosed in D1 (cf. Claim 1, p. 10, line 1 - p. 11, line 29, Examples 1 - 5). The wording "(transferring) tagged fragments" may be broadly interpreted as to include the exploitation of an inherent (human specific) tag sequence such as ALU sequences (for identification) which is to be expected to be present in fragment of larger than 10kb size (cf. the size limits given in claim 2). The specific meaning that is given to the wording "tagging" on p. 3, lines 19 - 23 is not present in claim 1. Thus, the claimed method differs from that of D1 mainly in that a syngeneic system, i.e.the tumour cell being transfected originating from the same (syngeneic) animal strain into which the transfected cells are injected and allowed to grow, is used.

The advantages correlated with a entirely syngeneic cell/host animal system in the finding and identification of metastasis inducing DNA were, however, already recognized prior to the priority date of the present application as is apparaent form D3 (see p. 2785, the Introduction).

This document describes the screening system (RAMA 37 cells/Wistar-Furth rats) as used in the present application (see the abstract). The method described in D3 solely differs from that according to claims 1, 2 4 and 5 in that no recovery of human DNA has been made.

Having regard to the improvements to be expected a skilled person would obviously replace the cell/animal system of D1 by that of D3. Moreover, extension of the method of D3 by an additional step for recovery of metastasis associated DNA from positive clones (such as disclosed in D1) is explicitly suggested in D3 (see the last sentence of the DIcussion, p. 2792).

Thereby one would automatically arrive at the subject-matter of any of claims 1, 2 4 and 5.

11. The use of a oligonucleotide tag as defined in claim 6 in order to aid insertion into and rescue from the host cell genome of DNA fragments in the method according to claim 1 is not anticipated by the prior art taken into consideration. Having regard to a possible effect of the tagging oligonucleotides on the metastatic potential of the DNA fragments to be transfected, this modification of the method as disclosed in any of D1 or D3 is not obvious for a skilled person.

    Thus, the subject-matter of claim 6 appears to satisfy the requirements of Art. 33(2) and (3) PCT.

12. The subject-matter of claim 14 lacks inventive step (Art. 33(3) PCT).

    According to D4 expression of osteopontin DNA has been associated with tumour progression and induction of metastasis formation. The test system disclosed in D4 (expression of osteopontin antisense RNA) confirm this hypothesis (see p. 832, right-hand column, lines 14 - 18, the final two phrases on p. 835, p. 837, last paragraph of the Discussion).

    Thus, in spite of the applicant's arguments, the use of osteopontin DNA as metastasis inducer is considered to be obvious for a skilled person in the light of the disclosure of D4.

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12Q 1/68, C12N 15/11 — A1

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(30) Priority Data:
9600470.0 10 January 1996 (10.01.96) GB

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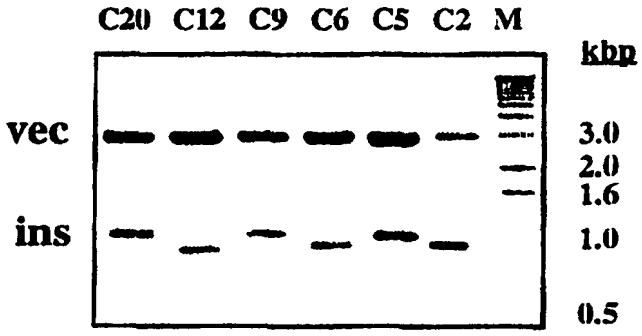
(81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METASTASIS INDUCING DNA'S

(57) Abstract

The invention relates to metastasis inducing DNA's, a method of identifying such DNA's and their use in diagnosis and therapy. It includes a method of screening and recovering Met-DNA comprising the steps of: (1) transferring fragments of human DNA from malignant, metastatic cancer cells into a cell line that produces only benign, non-metastasizing tumours when injected into a syngeneic animal; (2) injecting the transformed cells into a syngeneic animal; (3) selecting those animals in which metastasizing tumours have been identified; and (4) recovering the Met-DNA therefrom.



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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/00074

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68 C12N15/11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CANCER RESEARCH, vol. 54, 1994, pages 2785-2793, XP002032119 DAVIES ET AL.: "Induction of metastatic ability in a stably diploid benign rat mammary epithelial cell line by transfection with DNA from human malignant breast carcinoma cell lines"	1-4
Y	see the whole document ---	5,6,14
X	WO 94 28129 A (ISIS INNOVATION ;TARIN DAVID (GB)) 8 December 1994	1-4
Y	see the whole document ---	5,6,14
X	WO 86 03226 A (WHITEHEAD BIOMEDICAL INST) 5 June 1986	1-4
Y	see the whole document ---	5,6,14
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
2 June 1997	11.06.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Hagenmaier, S

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/GB 97/00074

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 607 054 A (HONJO TASUKU ;ONO PHARMACEUTICAL CO (JP)) 20 July 1994 see the whole document ---	5,6
Y	CANCER RESEARCH, vol. 54, 1994, pages 832-837, XP002032120 BEHREND ET AL.: "Reduced malignancy of ras-transformed NIH 3T3 cells expressing antisense osteopontin RNA" see the whole document -----	14

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

information on patent family members

Inte onal Application No
PCT/GB 97/00074

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9428129 A	08-12-94	AU 6802294 A	20-12-94
		EP 0700436 A	13-03-96
WO 8603226 A	05-06-86	AU 5197986 A	18-06-86
		EP 0203970 A	10-12-86
		JP 62501399 T	11-06-87
EP 0607054 A	20-07-94	CA 2113363 A	15-07-94
		JP 6315380 A	15-11-94
		US 5525486 A	11-06-96

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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GRANDE BRETAGNE

-9 MAR 1998

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Applicant's or agent's file reference JDM/DCS/P.93928WO	Date of mailing *(day/month/year)* 06.03.98 **IMPORTANT NOTIFICATION**
International application No. PCT/GB97/00074	International filing date *(day/month/year)* 10/01/1997
Priority date *(day/month/year)* 10/01/1996	
Applicant THE UNIVERSITY OF LIVERPOOL et al.	

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/ European Patent Office D-80298 Munich Tel. (+49-89) 2399-0. Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer Hebert, W Tel. (+49-89) 2399-8161

Form PCT/IPEA/416 (July 1992)

DESCRIPTION

METASTASIS INDUCING DNA'S

The present invention relates to metastasis inducing DNA's, a method of identifying such DNA's, and their use in diagnosis and therapy.

Most cancers are thought to be due to alterations in specific genes caused either by mutation making their gene-product in some way more effective or by over expression of a normal gene giving an enhanced effect. These oncogenes have largely been identified by introducing gene-length fragments of DNA from human cancers into a mouse fibroblast cell line, in culture, and selecting those cell lines that grow in an uncontrolled manner in liquid or semi-solid medium. The oncogenes themselves have been isolated by cloning the human DNA fragments away from the mouse DNA by standard recombinatorial techniques. Alternatively mutations can arise in genes that suppress their own activity such as, for example, p53 or Rb or which suppress the levels of their products such as, for example NM-23. These are referred to as tumour suppressor oncogenes. In the commonly-occurring cancers, it is believed that between 5 and 7 such changes in oncogenes or tumour suppressor oncogenes are required to produce a full-blown cancer.

WO 86/03226 discloses a method for detecting a discrete, transmissible mammalian gene associated with tumour metastasis. The method uses a non-syngeneic

system. The teaching was later retracted - Proc Nat. Acad. Sci USA, 1988, 85 5581.

WO 94/28129 identifies a tumour metastasis gene of 2858 base pairs which codes for a protein which is expressed in malignant human tumours and their metastasis. The method used to identify it used a non-syngeneic system employing nude (defective) mice.

Cancer research 54, 2785-2793 (1994) is a paper by the applicants. It discloses a method for showing the presence of metastasis inducing DNA. No disclosure is, however, made of how to recover the sequences for identification.

Cancer research 54 832-837 (1994) is a paper suggesting that antisense OPN DNA expression was associated with reduced tumorigenicity of these cells in the flanks and in lungs. The paper does not measure or investigate metastasis as such.

EP 0607054 disclosures a process for constructing a cDNA library. It described a method, using linkers and PCR for identifying signal peptides. The application is not to metastasis at all and the approach uses expression vectors for detection.

The major forms of cancer, including breast cancer, lung cancer and colonic cancer cannot be cured effectively because, although the current therapies may

invention there is provided a method of screening and recovering a regulatory DNA capable of inducing metastasis comprising the steps of:

i. transferring tagged fragments of a human DNA from malignant, metastatic cancer cells into a cell line that produces only benign, non-metastasizing tumours when injected into a syngeneic animal;

ii. injecting the transformed cells into the syngeneic animal;

iii. selecting those animals in which metastasizing tumours have been identified; and

iv. recovering the regulatory DNA capable of inducing metastasis therefrom.

Preferably the DNA fragments transferred in step 1 are fragments of from 0.1 to 50 kilo base-pairs, more preferably 0.5 to 50 kilo base-pairs.

Preferably the cell line that produces only benign non-metastasizing tumours when injected into a syngeneic animal is a rat mammary epithelial cell line, such as, for example Rama 37.

Preferably the fragments of human DNA from malignant, metastatic cancer cells are tagged to assist in their removal or insertion from or into a host or vector, such as, for example, the oligonucleotide tag illustrated in Fig. 1. This tagging procedure overcomes the problem of identifying the inserted human DNA sequences in the rat genome of the transfected rat cells. Human-specific repetitive DNA (Alu) sequences are spaced sufficiently in the human genome that in many human DNA

in pilot studies in the DNA of human breast cancers. Hybridisation of C9-DNA occurs to *Hind*III-digested DNA from 4 out of the 9 breast tumours tested, whereas no hybridisation signal is detected from similarly-digested DNA from normal human breast or colon tissue. In this case a single hybridising band of 1000bp is detected (Figure 6).

Figure 6 illustrates detection of C9-DNA in human breast tumours. Cellular DNA was isolated from a selection of nine randomly-picked human breast tumours numbered 14-130 and from normal breast and colon tissue together with C9-DNA as a control. These DNAs were digested with an excess of *Hind*III and the digested DNA was analysed on agarose gels, Southern blotted on to a filter and hybridised to a probe of [^{32}P]C9-DNA without tags and the radioactivity visualised on X-ray film. Similar results have been obtained using PCR for C9-DNA.

According to a second aspect of the present invention there is provided a regulatory DNA capable of inducing metastasis consisting essentially of a human DNA fragment of less than 1.6 kilobase pair in length obtained from a malignant, metastasis cancer cell.

According to a third aspect of the present invention there are provided DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 1:

C2

CTTCCTTGGT GCTCTATGTC TTGCCTCTCC CCTTCTCCAG TCCCATTAAG CCATAACCAT
CTTGACAGAC TCTGGGACAG TCCCCTCTGC TCTCCTGTTG GCGCCTGAGT CCCTTTTTGC
CTGAGGACCC TTCACGTAGC CTCCCATCTG GATGACCTAG TAGAAGACGT GGGAAGTTGT
CACACTCAGG TAACTGAGCA GAGCTCAGAG ATTTAAAGTG AGTCTGGGGA GCCTCGAGGA
TTGATCTGCT GCCTTAAAAA GCCAATTGGA TGACTAACCC AGACTATTGT CACTTTAGGT
GGGAAGTCAC TAGCATATCT GATGGGTCAC ATCTGAGAAA GGTTTCTAGC AGTGGTGGCC
TTGTGTGAGC AGCATGGCGT GTATCATGGT GTGCAGCATA CTCAGGCTGC TTGCAACACT
CGAGGCTCTT CTTCAGTATT AGGGGAACCA CTGGTGTTSG AACATGGTCC AAGAATACAG
TCATGTGAGG AGAATCCCAA TGCGTCAGGA GAAAACGAGA GTCTGTGACC TCCATTCTTC
AAGATACAGA ATTATTCTTG GACTGTGTTT TCATGCTCCT TGTGGATGGG AGTGAGTTTA
CTTCAGGTTA ATCAGCATTG CTTACTGTTG GTATTCAAGT AAATGCTTAA ATTATCCTGG
ATATACCTCT GTGGGAAGCA GGTTTTTGAT ACATGCAGCT TGTCCTTGTG ATTGATACTG
CTTGAACTCA AGAGAACTTT GCTCATGTGA TCTTTCTTAA CCGATGGAGT AGAAACTGTC
TGATGCTCTC AATAAAGTTG GCTCTTGCAC GAGACGTTAG TCTGTCCTGT TTATCTGCTC
CATTCTTCCG CTCCCACGGC CTCTACAGCA CTAAACCCAC CACCGATAGA CTCAGTCTTT
CACTGACAAA CATCACCAGA GGCTCTTAAC TGAGATTATA AACTGTTACT AGATGATGGG
TGGAATCGCT CCCCAGAAAC ATAAACATTT ACTTGGAGAA CTCAAGACCC CTTTGTAGAC
ATAACTCCCA TGGT

According to a fourth aspect of the present invention there are provided DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 2:

C5

ATTGCTGTGA GCCTATTAGC GACATTTGGT GACGCCCCTT TTAAGGGGGT AGATACAAAG
AATGGGTTGA AATTCTGTGC CACAAACGCT CTCCATGTTT TCACAATTAC ACTTGCAACC
TGTGGTCAGC AGCCAGAATT TAGGGATGTG ATGGGACAGG GTCGGGGAAA GAAGGAGAAG
GGTAAAGGAA AGACAGCACG TTAAAGTCCA AACAGCTCCA GGAGACTATC TGTAGAAATA
ACATCAGACC ATGAGGAGAA TTGATATCAT TGTTTTTCAA TGGGTATCGC CAAGGGAACT
TTCCATCTGA TTAAAAATAA TTACTGCTGG CACTAAATCC AATTGGAAAT GCCCCACACA
ATTTATCTTC CACTTCATGC TGCTACCATA TGCCTGACGT GGCGGAGCAG AAGCATTCCC
TCCCGTTCTG ATAAATAGTA CTTTGTAAAT ATTTGGAGAC GGGAGCTCTG GTGACAGGGA
ACACGTACAA ACCGGCCTGT TTATCATGTT CCCGATAGAG GCCCTCTTTG ACGTACAGGA
CCCCAAAACA GTCAGGATGC TGTGAATTTC CTTCCATGAA GCCTTGTTCA CAATTAGCAA
CCATTGGAGG AAGCAGGCTG CACTGTCTAC CACAAGTGGC ACTTTCCAAA GAGCACACAT
ATATTGGAGC AAGACATTTT GCTGGCTGAC TGGTGCTGTG TAAGCTGATA AACTGCTATA
TTTATTAAAC TGGCTTTTCT TTGAACACCC CACTCAAGGA AAAAAAAACA CACTTAGGGT
GACATTATTT GGAGATGAAG TCTTTATAGA GATGCTTAAG TTTAAACGAG ACTTTTAAAG
CCGGCTCTAT TCCATTTAAT GAATGGTGTC CCTACAAAGG AAGAAACTGG GACAGAGGTA
TGTACACTTG TGTGTGTGTG AGAGACAACG TGAGGAGCTG AAGAGGAGCA CGTACAAGTC
AGAGAAAGGC TGACCCTTAT TCACACTGAG CAAACCAGTC ATGTGTGGGT CGATAGATGA
GAGTATCCCC CAAGACTCAC ACATTCGAAC GCTTGGTC

AMENDED SHEET

According to a fifth aspect of the present invention there are provided DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 3:

C6

AGGACCAGAG TTCACATCCC ATCAAATGGC CCAGAAGGTT TTAATGCTGT CTTTTGGCCC
AGGGGCGAAC TGCACACACA TGTGCACATA CACTTACAGA GACACACATT CAGCAGCATA
AGAACACAAT CACAAATAAA AAAAATCTTG AAAAAATTTA AGCTAAAATT GTTAAGAAAT
AACATATACA CAATTTTTCT TTATTTTTTT AAAGATTTAT TTATTTAATG TATATGAGTA
CACTGCCTCT CCCTCCAGAC ATAGCAGTAC AGGGCATCGG ATCCCATTAC AGATGGTTGT
GAGCCACCAT GTGGTTTCAC AGATGGTTGT GAGCCACCAT GTGGTTTCAG GAATTGAACT
CAGGACCTTT GGAAGAGCAG TCAGTGCTCT TAACCTCTAA GCCATCTCTC CTGACCCTTA
TATACAATTT TAATGCTACG TACACACAAC TTCTCTTTCC TTTAATGGTT GAGATTTTTG
TCTGGAGAAG TAAGAATAAA GGAGGGAAAG AACATTGCTT TCACATTGCA CCAGTGGGAA
CAGCGTGTTT AAAGTAGGAA TGCCATGAAA TGACTGGCCT GCCTTCTCAT TACTGTTCCT
CCCACTCCTC CTTTTAACTG GAGCTCCTTT ATCTAATTTA TTAGTTTGAC GATACCCAGG
GTTTTCTTCT GTTTTGATCT TTTTAAGACA GAGACTCACC ATATAGCCCT GGCTGGCCTG
AAGCTCACTA TGTAGACCAG TCTGGCCTTG AACTCAAAGG AGATCTATCT GCTTCCTAGT
GCTGGGATTA AAGGCTTGTG CTACCAAGTC TGGTCTGAGG CTTTGGAGCA GCCTCGGTTT
TGGCCTTCTT TAAGGATCTC TAAGCTAGCA GTAAGTAGCC TAGCCATGCT GTTGTAGGAA
GTTGTTCGTT CATCCTGGCT CCAGCACAAA GGCAGTCACT AAACGTCGGC CTCATTTCAT
CAGAGCTGAA TGCAAATTCC TTGTGCTCTT CCTGTGTCCT CCTGGAAC

According to a sixth aspect of the present invention there are provided DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 4:

C9

AGTTGGGGAC ACAGCTTGCT TGATTAAGAT GTTTCTTGGG AAAAGGAGTT AAGCCTAATG
ATTTCCAATG GAAAGGACTG CTAATTGGGG AGGCAATGTT GCTTAATTGG GACACCTGCG
GGTAATTAAA AGCTCTCTCC CAGTGGCCTT TCCTGTTTTT GGCTCTGGGA GGCGAAGGCA
TTGAGAGGGA TGCAGGCATT CTAAGGGCTG GTTCTTGGTT TCTCCCTTCC CCTCTGTCCA
AACTCAGTGA GGTATCCCTG TCTGTGCTGT CCTTAGAGTG CCGTCCTGAG GCCTTGGTGA
GTTAAGGTCT CTGGATCTGA GCTGCCTCAG GGAAACGCAT GAGCTCATTG GAAAGGGGAG
AACCAGGCAA AGGTGTTGGC TGTGACCTCA GAATTCTGAG GGGCAAAGGT TCAAGGCTAA
CTCTCATTAT AGAGCAAGTT TGAGACTGGC CTGGGAACAA AAATATAAAG TGAGTGAGGT
CATATGACAG CACCTGAGGA GTCCTGTCCC TAGAGATCAT AAGGACCTGG CTGCTGGGGA
CTTGTTGCAG ATGGCACTTT GTGTCGAGAG AGGGGACCTG CCCCAGCATG GGAGGCCCTG
GAAGATCCTC TGGATTAACT GTGAACACTG ATTGCTGCTT TATACCTGGA GTTGTGCTGT
TATCTGGTAC ACATCTGCTG GGTGAATGAG TTCATGGGCT TTATTTCAGT GAGGTATTTA
CCTGAGGAGA AAGAAGGACT GGTGCCACAA AGCACAGCTT TTAAATCTGT GGGTTGTGAC
CCATTATGGA CTATCATAAC TGAGTGCAGG TATCAAGAAT ACTTTAGCAG GTGGTAAAAA
GATTTTTGAA TGCGCAACGA CCAAAACTGA ACTCAAAAAT CAAGCATGGC ATGGATCCTG
GGTGCTCCTG GAAGCACTTG CCTTTACTGC ATTGTGCGAC TTGACGGTAG CCTTGGTTCT
GAATGCACAA CACGTGGGCT TTGGGCTGCA CAGGCCACCA CGCCGTGCCT GAAACACCTC
AGCTCAGGTT TGTGGCTATG TCCTATGACT TGGACTTACT TTTATTGCAC ATATAAATAT
TTTCCTGC

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According to a seventh aspect of the present invention there are provided DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 5:

C12

GAGGGGGTGG TGGCACAGTT ATGTTTTTGT AGGAAGGGTT CCATGAACCT CAGCAGAGCT
CGGGTTAGAA ATTTAAAAGC CCTGAGGGGA ATTTTTTTTT TAAATCGCTA TGAATCTGAC
ATGAGAAAAA CAGATCAGAA ACGTTCTTGT GCTTCAGAAA AGGACAAGTG TGTGAGCTAA
CAGACTGCAC ACTGGTGTTC GAGGCACATC TGGATCACAG GAGCGTCAGA TAATGTCCCC
AAAGGTAAAT GCATTTGCTT GCACAGTACC GAGTGTGGTG GGGGGTGCCT ACAGCCCAGC
GGTTCTCAAC CTTCCTGATG CTTCGACCCT TTAATACAGT GCCTCATGCT CTGGTGACCT
CCCCAACCTT AAAATTATTT TTGTTGCTGT TCATAACTGT GATTTTGATA CTGTTATGAA
TTGTAATATA AATAATTTTG AAGAAAGAGG TTTGCCAAGG GTTTGAGAAC TGCTGTTCTA
GCCCCACGTG GATGGTTTTT CGTCATTTGG GGTTTTTATG AGGCAGAGTC TTATGTAGCC
CAGGCTAGCA GCCTAGAATG TGCTACTTAG CTGAGGAATA ACCTTGGAAC TTCTGAGGAC
TGGAGAGACT GGCTTAGTCC TCAAGAAACT GGAAATAGCT GGAGTTTGGC TACTTGTGGG
TTCCTTTTTC TTCAAACCTT TTCTACTCTT TTTCCACCCT GTCGGCCCCC TAACACTAAA
TAAGAAAGAG AAAGGGGAGC ATAGAGGGGA AAAGAAACCC CTGAATAACG TCAGTAGTTG
GCAAAGGGGG GTGACATATG TTGTCATTAG ACCACATCCT GGTGATTAAG GGGAGTCAAG
TTCCTTGGGG CAAGTTTGAT CTTTCGTGTA ACGATATCTA ATTTCTTCTC CCTGTTGCTT
CGTCTTTGTG AACAACGACT TGATAACCCA CAATGGACCA TCAACCAACC AACCAACCAT

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According to a eighth aspect of the present invention there are provided DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 6:

C20

TGGTCTCTGG TGTTACTTGT TTTCCCATTT CTGACAGTGG TTTGACCTT CTATACGCCT
GTGTGTCAGG AGTGCTGTAG ACCTATTTTC CTGTTTTCTT TCAGCCAGTT ACAGGAACAG
AGTGTTCTAC TGTCAGATGT GTAGCTGTTC CTGTCCACTG ACTTTCAAGC TGTCTCTGTG
TGCAGGAACC AGAAGGGCCT GTCCCTACTT CTACTGGGCC CCTACGCACA GGGGGCCTAG
ATGGTGCTAG GTGTTTTCCT CTAGAGCCTG AAATGTGGGC AGAGAGTAGT CTCCTCTGGT
TTCCTAGGTA TGTCTTCCCC TCTGAAGGTC TAGCTCTCCC TTCCATGGGA TATGGGTGCA
GGGAGCTGTT TGACCAGGTC CTCTCAAATC CGGGTGCAGT CTGGACCGCA GGCTCCTGTA
GCTTGCCTGC TGCAATCTTC CCGCACCCAG AGGCACCCAA GTTTCCTCTT GGGCCAAGGA
TGTGGGCAAA GGTGGGCAGA AGTGGCAATC TCTCCTGCCC TAGCGTCTCA GGATTGCCCT
CACTTCTGGG CAATCCGCTC TCTCTTCCAC AGGGTTTGGG AGCAGGGAGC TGTGGGCCGG
TATCAGGCAA AGGTTTGAGG CAACCAGTTA GAAACTGGAA GTGTCAGGTC CCAGAGGAAT
TTTGCCTTTG TGTGTCCTGA GTCCACCAGG CAGGTCACTT GGAGCAGAAA AATTGGTTTT
CCCCTCGGTC TCAGGCCTGA AGTTGCACCT CAGGGTTGGC TTTCAGCTGT ACCTGTGGAA
AGTATGGTTT TAAAAATCTA AGATAGCTAT CATGCAGCAA GGCTTGTGTA AAATGTCTAT
TTGGTTCCTT TATGACTTAC TTTTGCTGTA CTGAGGATCA AACCTAGGGT CTCAAGCAGT
CATCACAATT CTCTGTCACT GATCCAGCTC CATTTCTATT TTCTTTTGTC CCGCGCGATC
TCTCGCCAGC AAGAAAACAC GCTAGGGACA TACGAATCCT TGCTGCAGCC AAAACTTTTA
TTGAATCTTA AGGAGAAGCC CGCGCACCGG ACTGGCGCGG TTTATATACA CCCTAGCACA
GTGCATCCAC A

Detailed examination of their DNA sequences has confirmed that the six Met-DNA's bear little relationship to one another. C6-DNA shows 86% homology to 102 bp of the rat WAP promoter (Nucleic Acids Res. 12 8685-8697 1984) with a novel duplication of 30 nucleotides of this region. All Met-DNAs contain recognition sequences for transcription factors TCF-1 (EMBO J. 10. 123-132, 1991) and HIP1b (Mol.cell. Biol. 10, 653-661, 1990). Moreover all but one contain recognition sequences for CTCF (Oncogene 5, 1743-1753, 1990), HIP1a (Mol.Cell.Biol.10, 653-661, 1990), NF-1L6 (EMBO J. 9 457-465, 1990) and regions of potential Z-DNA (Nature 282, 680-686, 1979),

with C6-DNA containing a tract of 23 alternating purine-pyrimidine bases. Thus these novel sequences all contain potential regulatory regions for transcription of DNA into mRNA but no known coding or viral-related sequences.

According to an ninth aspect of the present invention there is provided the use of an osteopontin gene as a metastasis inducing transformant.

In one embodiment Met-DNA's, are introduced into a benign rat mammary epithelial cell line Rama 37.

By way of example and to help identify the regulatory function that short stretches of human malignant DNA (precursor to Met-DNA's) may exert on the transfected Rama 37 cells, the mRNA expression of the metastatic transformant rat mammary cell line R37-Ca2-LT1 was compared with its benign parental cell line Rama 37 using subtractive hybridisation techniques. Of the four subtracted clones three corresponded to known rat genes for proteins including osteopontin and one corresponded to a novel rat gene of unknown function. As an example only, transfection of rat osteopontin cDNA into the parental Rama 37 cells produced transformants that induced a high frequency of metastasis compared with vector controls confirming the metastatic capability of

invention there is provided a probe specific to a regulatory DNA capable of inducing metastasis.

By specific is meant hybridises to any target DNA under suitable salt and temperature conditions to allow detection of identical or related DNA molecules.

Preferably the probe is provided as part of a kit which may additionally comprise one or more of the following: a colour indicator; an oligonucleotide primer; materials for gel analysis, and/or materials for DNA transfer or hybridisation.

The Met-DNA sequences may be detected in tumour or biopsy specimens by standard Southern blotting, PCR-based or in-situ techniques to identify those patients at risk from metastatic disease. Physical methods of detection based on imaging techniques may also be possible. Expression of metastasis - inducing genes may be detected by standard mRNA hybridisation PCR amplification or by antibodies specific for the gene-product.

According to a eleventh aspect of the present invention there is provided a medicament adapted to target a regulatory DNA capable of inducing metastasis as claimed in any of claims 7 to 13.

In one embodiment such Met-DNA's, metastasis-inducing genes or fragments thereof, could be

CLAIMS

1. A method of screening and recovering a regulatory DNA capable of inducing metastasis comprising the steps of:

i. transferring tagged fragments of a human DNA from malignant, metastatic cancer cells into a cell line that produces only benign, non-metastasizing tumours when injected into a syngeneic animal;

ii. injecting the transformed cells into the syngeneic animal;

iii. selecting those animals in which metastasizing tumours have been identified; and

iv. recovering the regulatory DNA capable of inducing metastasis therefrom.

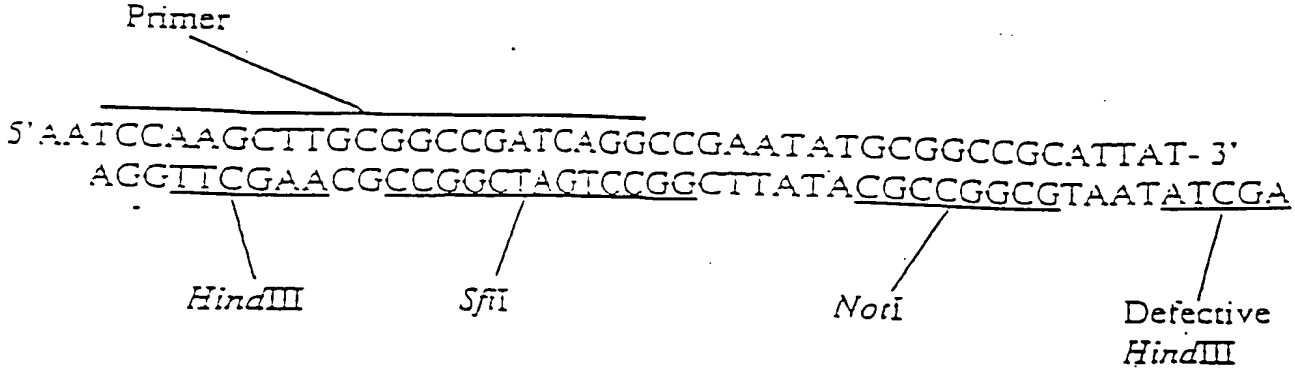
2. A method as claimed in claim 1 in which the fragments of human DNA transferred in step 1 are from 0.1 to 50 kilo base pairs in length.

3. A method as claimed in claim 2 in which the fragments of human DNA transferred in step (i) are less than 1.6 kilo base pairs in length.

4. A method as claimed in claim 1, 2 or 3 in which the cell line that produces only benign non-metastasizing tumours is a rat mammary epithelial cell line.

5. A method as claimed in claim 4 wherein the rat mammary epithelial cell line is a Rama 37 cell line.

6. A method as claimed in claim 5 wherein the tag is an oligonucleotide sequence:



AMENDED SHEET

7. A regulatory DNA capable of inducing metastasis consisting essentially of a human DNA fragment of less than 1.6 kilobase pair in length obtained from a malignant, metastasis cancer cell.

8. DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 1:

C2

CTTCCTTGGT GCTCTATGTC TTGCCTCTCC CCTTCTCCAG TCCCATTAAG GCATAACCAT
CTTGACAGAC TCTGGGACAG TCCCCTCTGC TCTCCTGTTG GCGCCTGAGT CCCTTTTTGC
CTGAGGACCC TTCACGTAGC CTCCCATCTG GATGACCTAG TAGAAGACGT GGGAAGTTGT
CACACTCAGG TAACTGAGCA GAGCTCAGAG ATTTAAAGTG AGTCTGGGGA GCCTCGAGGA
TTGATCTGCT GCCTTAAAAA GCCAATTGGA TGACTAACCC AGACTATTGT CACTTTAGGT
GGGAAGTCAC TAGCATATCT GATGGGTCAC ATCTGAGAAA GGTTTCTAGC AGTGGTGGCC
TTGTGTGAGC AGCATGGCGT GTATCATGGT GTGCAGCATA CTCAGGCTGC TTGCAACACT
CGAGGCTCTT CTTCAGTATT AGGGGAACCA CTGGTGTTSG AACATGGTCC AAGAATACAG
TCATGTGAGG AGAATCCCAA TGCGTCAGGA GAAAACGAGA GTCTGTGACC TCCATTCTTC
AAGATACAGA ATTATTCTTG GACTGTGTTT TCATGCTCCT TGTGGATGGG AGTGAGTTTA
CTTCAGGTTA ATCAGCATTG CTTACTGTTG GTATTCAAGT AAATGCTTAA ATTATCCTGG
ATATACCTCT GTGGGAAGCA GGTTTTTGAT ACATGCAGCT TGTCCTTGTG ATTGATACTG
CTTGAACTCA AGAGAACTTT GCTCATGTGA TCTTTCTTAA CCGATGGAGT AGAAACTGTC
TGATGCTCTC AATAAAGTTG GCTCTTGCAC GAGACGTTAG TCTGTCCTGT TTATCTGCTC
CATTCTTCCG CTCCCACGGC CTCTACAGCA CTAAACCCAC CACCGATAGA CTCAGTCTTT
CACTGACAAA CATCACCAGA GGCTCTTAAC TGAGATTATA AACTGTTACT AGATGATGGG
TGGAATCGCT CCCCAGAAAC ATAAACATTT ACTTGGAGAA CTCAAGACCC CTTTGTAGAC
ATAACTCCCA TGGT

9. DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 2:

C5

ATTGCTGTGA GCCTATTAGC GACATTTGGT GACGCCCCTT TTAAGGGGGT AGATACAAAG
AATGGGTTGA AATTCTGTGC CACAAACGCT CTCCATGTTT TCACAATTAC ACTTGCAACC
TGTGGTCAGC AGCCAGAATT TAGGGATGTG ATGGGACAGG GTCGGGGAAA GAAGGAGAAG
GGTAAAGGAA AGACAGCACG TTAAAGTCCA AACAGCTCCA GGAGACTATC TGTAGAAATA
ACATCAGACC ATGAGGAGAA TTGATATCAT TGTTTTTCAA TGGGTATCGC CAAGGGAACT
TTCCATCTGA TTAAAAATAA TTACTGCTGG CACTAAATCC AATTGGAAAT GCCCCACACA
ATTTATCTTC CACTTCATGC TGCTACCATA TGCCTGACGT GGCGGAGCAG AAGCATTCCC
TCCCGTTCTG ATAAATAGTA CTTTGTAAAT ATTTGGAGAC GGGAGCTCTG GTGACAGGGA
ACACGTACAA ACCGGCCTGT TTATCATGTT CCCGATAGAG GCCCTCTTTG ACGTACAGGA
CCCCAAAACA GTCAGGATGC TGTGAATTTC CTTCCATGAA GCCTTGTTCA CAATTAGCAA
CCATTGGAGG AAGCAGGCTG CACTGTCTAC CACAAGTGGC ACTTTCCAAA GAGCACACAT
ATATTGGAGC AAGACATTTT GCTGGCTGAC TGGTGCTGTG TAAGCTGATA AACTGCTATA
TTTATTAAAC TGGCTTTTCT TTGAACACCC CACTCAAGGA AAAAAAAACA CACTTAGGGT
GACATTATTT GGAGATGAAG TCTTTATAGA GATGCTTAAG TTTAAACGAG ACTTTTAAAG
CCGGCTCTAT TCCATTTAAT GAATGGTGTC CCTACAAAGG AAGAAACTGG GACAGAGGTA
TGTACACTTG TGTGTGTGTG AGAGACAACG TGAGGAGCTG AAGAGGAGCA CGTACAAGTC
AGAGAAAGGC TGACCCTTAT TCACACTGAG CAAACCAGTC ATGTGTGGGT CGATAGATGA
GAGTATCCCC CAAGACTCAC ACATTCGAAC GCTTGGTC

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10. DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 3:

C6

AGGACCAGAG TTCACATCCC ATCAAATGGC CCAGAAGGTT TTAATGCTGT CTTTTGGCC
AGGGGCGAAC TGCACACACA TGTGCACATA CACTTACAGA GACACACATT CAGCAGCAT
AGAACACAAT CACAAATAAA AAAAATCTTG AAAAATTTTA AGCTAAAATT GTTAAGAAA
AACATATATA CAATTTTTCT TTATTTTTTT AAAGATTTAT TTATTTAATG TATATGAGT
CACTGCCTCT CCCTCCAGAC ATAGCAGTAC AGGGCATCGG ATCCCATTAC AGATGGTTG
GAGCCACCAT GTGGTTTCAC AGATGGTTGT GAGCCACCAT GTGGTTTCAG GAATTGAAC
GAGGACCTTT GGAAGAGCAG TCAGTGCTCT TAACCTCTAA GCCATCTCTC CTGACCCTT
TATACAATTT TAATGCTACG TACACACAAC TTCTCTTTCC TTTAATGGTT GAGATTTTT
TCTGGAGAAG TAAGAATAAA GGAGGGAAAG AACATTGCTT TCACATTGCA CCAGTGGGA
CAGCGTGTTT AAAGTAGGAA TGCCATGAAA TGACTGGCCT GCCTTCTCAT TACTGTTCC
CCCACTCCTC CTTTTAACTG GAGCTCCTTT ATCTAATTTA TTAGTTTGAC GATACCCAG
GTTTTCTTCT GTTTTGATCT TTTTAAGACA GAGACTCACC ATATAGCCCT GGCTGGCCTG
AAGCTCACTA TGTAGACCAG TCTGGCCTTG AACTCAAAGG AGATCTATCT GCTTCCTAGT
GCTGGGATTA AAGGCTTGTG CTACCAAGTC TGGTCTGAGG CTTTGGAGCA GCCTCGGTTT
TGGCCTTCTT TAAGGATCTC TAAGCTAGCA GTAAGTAGCC TAGCCATGCT GTTGTAGGAA
GTTGTTCGTT CATCCTGGCT CCAGCACAAA GGCAGTCACT AAACGTCGGC CTCATTTCAT
CAGAGCTGAA TGCAAATTCC TTGTGCTCTT CCTGTGTCCT CCTGGAAC

11. DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 4:

C9

AGTTGGGGAC ACAGCTTGCT TGATTAAGAT GTTTCTTGGG AAAAGGAGTT AAGCCTAATG
ATTTCCAATG GAAAGGACTG CTAATTGGGG AGGCAATGTT GCTTAATTGG GACACCTGCG
GGTAATTAAA AGCTCTCTCC CAGTGGCCTT TCCTGTTTTT GGCTCTGGGA GGCGAAGGCA
TTGAGAGGGA TGCAGGCATT CTAAGGGCTG GTTCTTGGTT TCTCCCTTCC CCTCTGTCCA
AACTCAGTGA GGTATCCCTG TCTGTGCTGT CCTTAGAGTG CCGTCCTGAG GCCTTGGTGA
GTTAAGGTCT CTGGATCTGA GCTGCCTCAG GGAAACGCAT GAGCTCATTG GAAAGGGGAG
AACCAGGCAA AGGTGTTGGC TGTGACCTCA GAATTCTGAG GGGCAAAGGT TCAAGGCTAA
CTCTCATTAT AGAGCAAGTT TGAGACTGGC CTGGGAACAA AAATATAAAG TGAGTGAGGT
CATATGACAG CACCTGAGGA GTCCTGTCCC TAGAGATCAT AAGGACCTGG CTGCTGGGGA
CTTGTTGCAG ATGGCACTTT GTGTCGAGAG AGGGGACCTG CCCCAGCATG GGAGGCCCTG
GAAGATCCTC TGGATTAACT GTGAACACTG ATTGCTGCTT TATACCTGGA GTTGTGCTGT
TATCTGGTAC ACATCTGCTG GGTGAATGAG TTCATGGGCT TTATTTCAGT GAGGTATTTA
CCTGAGGAGA AAGAAGGACT GGTGCCACAA AGCACAGCTT TTAAATCTGT GGGTTGTGAC
CCATTATGGA CTATCATAAC TGAGTGCAGG TATCAAGAAT ACTTTAGCAG GTGGTAAAAA
GATTTTTGAA TGCGCAACGA CCAAAACTGA ACTCAAAAAT CAAGCATGGC ATGGATCCTG
GGTGCTCCTG GAAGCACTTG CCTTTACTGC ATTGTGCGAC TTGACGGTAG CCTTGGTTCT
GAATGCACAA CACGTGGGCT TTGGGCTGCA CAGGCCACCA CGCCGTGCCT GAAACACCTC
AGCTCAGGTT TGTGGCTATG TCCTATGACT TGGACTTACT TTTATTGCAC ATATAAATAT
TTTCCTGC

12. DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 5:

C12

GAGGGGGTGG TGGCACAGTT ATGTTTTTGT AGGAAGGGTT CCATGAACCT CAGCAGAGCT
CGGGTTAGAA ATTTAAAAGC CCTGAGGGGA ATTTTTTTTT TAAATCGCTA TGAATCTGAC
ATGAGAAAAA CAGATCAGAA ACGTTCTTGT GCTTCAGAAA AGGACAAGTG TGTGAGCTAA
CAGACTGCAC ACTGGTGTTC GAGGCACATC TGGATCACAG GAGCGTCAGA TAATGTCCCC
AAAGGTAAAT GCATTTGCTT GCACAGTACC GAGTGTGGTG GGGGGTGCCT ACAGCCCAGC
GGTTCTCAAC CTTCCTGATG CTTCGACCCT TTAATACAGT GCCTCATGCT CTGGTGACCT
CCCCAACCTT AAAATTATTT TTGTTGCTGT TCATAACTGT GATTTTGATA CTGTTATGAA
TTGTAATATA AATAATTTTG AAGAAAGAGG TTTGCCAAGG GTTTGAGAAC TGCTGTTCTA
GCCCCACGTG GATGGTTTTT CGTCATTTGG GGTTTTTATG AGGCAGAGTC TTATGTAGCC
CAGGCTAGCA GCCTAGAATG TGCTACTTAG CTGAGGAATA ACCTTGGAAC TTCTGAGGAC
TGGAGAGACT GGCTTAGTCC TCAAGAAACT GGAAATAGCT GGAGTTTGGC TACTTGTGGG
TTCCTTTTTC TTCAAACCTT TTCTACTCTT TTTCCACCCT GTCGGCCCCC TAACACTAAA
TAAGAAAGAG AAAGGGGAGC ATAGAGGGGA AAAGAAACCC CTGAATAACG TCAGTAGTTG
GCAAAGGGGG GTGACATATG TTGTCATTAG ACCACATCCT GGTGATTAAG GGGAGTCAAG
TTCCTTGGGG CAAGTTTGAT CTTTCGTGTA ACGATATCTA ATTTCTTCTC CCTGTTGCTT
CGTCTTTGTG AACAACGACT TGATAACCCA CAATGGACCA TCAACCAACC AACCAACCAT

13. DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 6:

C20

TTGTCTCTGG TGTTACTTGT TTTCCCATTT CTGACAGTGG TTTGACCTT CTATACGCCT
GTGTGTCAGG AGTGCTGTAG ACCTATTTTC CTGTTTTCTT TCAGCCAGTT ACAGGAACAG
AGTGTTCTAC TGTCAGATGT GTAGCTGTTC CTGTCCACTG ACTTTCAAGC TGTCTCTGTG
TGCAGGAACC AGAAGGGCCT GTCCCTACTT CTACTGGGCC CCTACGCACA GGGGGCCTAG
ATGGTGCTAG GTGTTTTCCT CTAGAGCCTG AAATGTGGGC AGAGAGTAGT CTCCTCTGGT
TTCCTAGGTA TGTCTTCCCC TCTGAAGGTC TAGCTCTCCC TTCCATGGGA TATGGGTGCA
GGGAGCTGTT TGACCAGGTC CTCTCAAATC CGGGTGCAGT CTGGACCGCA GGCTCCTGTA
GCTTGCCTGC TGCAATCTTC CCGCACCCAG AGGCACCCAA GTTTCCTCTT GGGCCAAGGA
TGTGGGCAAA GGTGGGCAGA AGTGGCAATC TCTCCTGCCC TAGCGTCTCA GGATTGCCCT
CACTTCTGGG CAATCCGCTC TCTCTTCCAC AGGGTTTGGG AGCAGGGAGC TGTGGGCCGG
TATCAGGCAA AGGTTTGAGG CAACCAGTTA GAAACTGGAA GTGTCAGGTC CCAGAGGAAT
TTTGCCTTTG TGTGTCCTGA GTCCACCAGG CAGGTCACTT GGAGCAGAAA AATTGGTTTT
CCCCTCGGTC TCAGGCCTGA AGTTGCACCT CAGGGTTGGC TTTCAGCTGT ACCTGTGGAA
AGTATGGTTT TAAAAATCTA AGATAGCTAT CATGCAGCAA GGCTTGTGTA AAATGTCTAT
TTGGTTCCTT TATGACTTAC TTTTGCTGTA CTGAGGATCA AACCTAGGGT CTCAAGCAGT
CATCACAATT CTCTGTCACT GATCCAGCTC CATTTCTATT TTCTTTTGTC CCGCGCGATC
TCTCGCCAGC AAGAAAACAC GCTAGGGACA TACGAATCCT TGCTGCAGCC AAAACTTTTA
TTGAATCTTA AGGAGAAGCC CGCGCACCGG ACTGGCGCGG TTTATATACA CCCTAGCACA
GTGCATCCAC A

14. The use of an osteopontin gene as a metastasis inducing transformant.

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15. A probe specific to a regulatory DNA capable of inducing metastasis as claimed in any of claims 7 to 13.

16. A kit for diagnosing the likelihood of a cancer metastasizing comprising a probe as claimed in claim 15 and one or more of a colour indicator, an oligonucleotide primer, materials for gel analysis and materials for DNA transfer or hydridisation.

17. A medicament adapted to target a regulatory DNA capable of inducing metastasis as claimed in any of claims 7 to 13.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
JDM/P93928WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 97/00074	10/01/1997	10/01/1996
Applicant		
THE UNIVERSITY OF LIVERPOOL et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [X] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [X] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. 3
   - [X] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/00074

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68 C12N15/11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CANCER RESEARCH, vol. 54, 1994, pages 2785-2793, XP002032119 DAVIES ET AL.: "Induction of metastatic ability in a stably diploid benign rat mammary epithelial cell line by transfection with DNA from human malignant breast carcinoma cell lines"	1-4
Y	see the whole document	5,6,14
X	WO 94 28129 A (ISIS INNOVATION ;TARIN DAVID (GB)) 8 December 1994	1-4
Y	see the whole document	5,6,14
X	WO 86 03226 A (WHITEHEAD BIOMEDICAL INST) 5 June 1986	1-4
Y	see the whole document	5,6,14

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
2 June 1997	11. 06. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hagenmaier, S

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 607 054 A (HONJO TASUKU ;ONO PHARMACEUTICAL CO (JP)) 20 July 1994 see the whole document ---	5,6
Y	CANCER RESEARCH, vol. 54, 1994, pages 832-837, XP002032120 BEHREND ET AL.: "Reduced malignancy of ras-transformed NIH 3T3 cells expressing antisense osteopontin RNA" see the whole document -----	14

INTERNATIONAL SEARCH REPORT

[redacted]nation on patent family members

[redacted]rnational Application No
PCT/GB 97/00074

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9428129 A	08-12-94	AU 6802294 A	20-12-94
		EP 0700436 A	13-03-96
WO 8603226 A	05-06-86	AU 5197986 A	18-06-86
		EP 0203970 A	10-12-86
		JP 62501399 T	11-06-87
EP 0607054 A	20-07-94	CA 2113363 A	15-07-94
		JP 6315380 A	15-11-94
		US 5525486 A	11-06-96